STANDARD TESTS TO CHARACTERIZE PEST RESISTANCE IN ALFALFA VARIETIES

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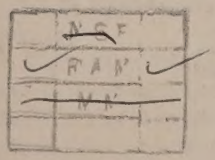

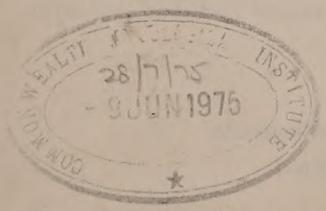

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PREFACE

This publication presents information assembled by the Alfalfa Improvement Conference Committee on "Standard tests for characterizing disease and insect resistance of alfalfa varieties". The report was approved by the 23d Alfalfa Improvement Conference held July 10-12, 1972, at Ottawa, Canada. Members of the committee and authors of the report are:

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The committee was assigned to: a) review the available information on evaluating pest resistance in alfalfa varieties and determine if information gaps exist, b) identify and describe pest evaluation procedures that could be considered as standard tests, and c) identify persons and locations that can provide information about variety evaluation tests for specific types of pest resistance.

The committee assembled this information for use as a working document rather than as a complete review of pest resistance research in alfalfa. It is intended for personnel concerned with breeding for alfalfa-pest resistance, crop production, and extension or marketing. The Agricultural Research Service, U.S. Department of Agriculture, published this report in cooperation with the Arizona, California, Kansas, and Minnesota Agricultural Experiment Stations.

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STANDARD TESTS TO CHARACTERIZE PEST RESISTANCE IN ALFALFA VARIETIES

NEED TO STANDARDIZE PEST RESISTANCE EVALUATION

Combined annual losses from diseases, insects, and nematodes amount to about 40 percent of the national alfalfa production. Developing varieties resistant to alfalfa pests has been an important contribution to agriculture. Most alfalfa acreage in the United States now is planted with varieties that were selected for resistance to one or more pests.

The importance of pest resistant alfalfa was recognized in 1942 with the introduction of the first two bacterial wilt-resistant varieties—Ranger for the northern Great Plains area, and Buffalo for the central Plains area. Stem nematode, spotted alfalfa aphid, and pea aphid resistant varieties followed and more recently alfalfa weevil, anthracnose, and *Phytophthora* resistant varieties were developed.

Recent worldwide protein shortages have suddenly shifted attention to using leaf-protein. Alfalfa is considered the most economical source of leaf-protein among all presently grown crops. However, to produce the maximum amount of high quality protein, growers will need to use pest resistant varieties. Therefore, growers, extension personnel, marketing specialists, and research scientists need uniform procedures to describe levels of pest resistance. Accurate descriptions of pest resistance levels also can be useful in characterizing varieties. The Plant Variety Protection Act gives special emphasis to the need for standardizing the characterization of pest resistance.

The goal of nearly all alfalfa breeding programs is to develop new varieties with multiple pest resistance. However, there are many pests and variety development takes time. Two problems alfalfa breeders face are the priority to assign to each type of resistance and the most effective method to increase resistance. This report presents a summary of pest resistance information for the purpose of helping the alfalfa industry to develop and to describe pest resistant varieties.

CHARACTERIZING DISEASE RESISTANCE

Background

Plant diseases may be caused either by abiotic agents or biotic agents. Abiotic agents include nutrient deficiencies, toxicities, physiogenic spotting, and air pollution damage . Differences occur among varieties in their reaction to some abiotic agents. The selection of strains resistant to air pollution by Howell, Devine, and Hanson (*26*)[1] is an example. In general, little progress has been made in developing resistance to abiotic agents. Therefore, the report is confined to diseases caused by biotic agents.

Biotic agents that incite diseases in alfalfa are bacteria, fungi viruses, and mycoplasmas. Graham, Kreitlow, and Faulkner (*15*) reported that at least 70 different pathogens occur on alfalfa and about 30 are considered limiting the growth and production of the crop. A survey of the United States by the committee, identified 22 diseases that were problems in more than one state. Fifteen diseases were identified as problems in enough states so that national distribution and severity maps could be developed.

Some research information is available on nearly every important disease in the United States, but often only one scientist may have worked with a pathogen. Even though information is available and variation in host-plant resistance has been reported for most important diseases, reliable evaluation procedures are available for only bacterial wilt, anthracnose, common

[1]Italic numbers in parenthesis refer to Literature Cited, p. 22.

leafspot, downy mildew, and *Phytophthora* root rot.

Sufficient information is available to screen for resistance to bacterial leaf spot, lepto leaf spot, *Rhizoctonia* crown rot, rust, *Sclerotinia* crown and stem rot, spring blackstem, *Stemphylium* leaf spot, summer blackstem, yellow leaf blotch, and alfalfa mosaic virus.

Unfortunately, procedures used to determine resistance to these pathogens usually rely on natural field epiphytotics or the pathogens have a number of biotypes, or both. Reliable evaluation procedures are not available except on occasion in the field. It may be possible with additional research to develop evaluation procedures for most, if not all, important pathogens in alfalfa.

Reliable evaluation procedures require that uniform infections can be repeated in subsequent tests. Percentage resistant plants and average severity index (ASI) should both be used to describe varietal reaction to diseases. Variety evaluations should be based on individual plant scores rather than on solid seeded plots. A 1 to 5 rating when feasible (1 = no disease, 5 = very severely diseased) is favored. Ratings for each pathogen should be based on specific lesion types or degree of infection (some diseases may require fewer or more than 5 classes). Rating values should be consistent from test to test. Generally, at least 100 plants should be used for evaluating a variety. Resistant and susceptible checks should be included in every test. The susceptible variety can be used to estimate percentage of escapes in a test. The variety being evaluated should be compared to the resistant check. Standard seed lots (when available) of the check varieties should be used. No agreement was reached on the number of tests required for evaluation. Individual scientists should decide this based on the number of escapes and relative precision of their data.

When describing the relative disease resistance of alfalfa varieties, the word "tolerant" should be used to denote a type of host pathogen interaction and not be used to denote low levels of disease resistance. The term "immune" probably will have no place in alfalfa variety descriptions, because of the difficulty with reaching homozygosity for any trait.

Summary of resistance information by pathogen species

Distribution and severity maps for 16 of the most important diseases in the United States appear on pages 9 to 11. The maps were based on observations and opinions of research and extension specialists from nearly every state. Scientists with expertise on specific pathogens are identified as resource people who can supply additional information about developing and evaluating specific types of disease resistance in alfalfa. Check varieties were identified for disease evaluations where sufficient information was available. In nearly all instances public varieties have been designated as checks instead of proprietory varieties. This was done to insure the availability of seed after the varieties are no longer commercially available. An attempt was also made to keep the number of check varieties to a minimum. For these reasons some varieties with higher levels of resistance and more extensive distribution may not have been selected as checks. The availability and type of screening and evaluation procedures (L = laboratory, F = field tests) have also been indicated.

BACTERIAL LEAF SPOT, *Xanthomonas alfalfae* (Riker, Jones & Davis) Dows.
Distribution and severity map: Figure 1
[1]Scientists and locations with expertise: 36 and 38

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	—
Nondormant types:	—	—

Screening procedures: L Evaluation procedures: none

BACTERIAL WILT, *Corynebacterium insidiosum* (McCull) H. L. Jens.
Distribution and severity map: Figure 2
Scientists and locations with expertise: 1, 10, and 41.

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	Vernal	Narragansett
Nondormant types:	—	Sonora

Screening procedures: L and F Evaluation procedures: F (page 13)

[1]Key for names of scientists that can serve as resource personnel listed on page 8.

ANTHRACNOSE, *Colletotrichum trifolii* Bain

Distribution and severity map: Figure 3
Scientists and locations with expertise: 2, 13, 16, and 27

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	Arc	Saranac
Nondormant types:	—	—

Screening procedures: L
Evaluation procedures: L (page 14)

COMMON LEAF SPOT, *Pseudopeziza medicaginis* (Lib.) Sacc.

Distribution and severity map: Figure 4
Scientists and locations with expertise: 11, 14, and 21

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	MSA-CW3AN3, Ramsey	Ranger
Nondormant types:	—	—

Screening procedures: L and F
Evaluation procedures: L and F (page 14)

DOWNY MILDEW, *Peronospora trifoliorum* de By.

Distribution and severity map: Figure 5
Scientists and locations with expertise: 1, 36, and 38

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	Saranac	Kanza
Nondormant types:	Caliverde, Mesa-Sirsa	Moapa 69

Screening procedures: L and F
Evaluation procedures: L and F (page 15)

FUSARIUM WILT, *Fusarium oxysporum* Schlecht f. *medicaginis* (Weimer) Snyd. & Hans.

Distribution and severity map: Figure 6
Scientists and locations with expertise: 9 and 16

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	—
Nondormant types:	—	—

Screening procedures: none
Evaluation procedures: none

FUSARIUM ROOT AND CROWN ROT, *Fusarium spp.*

Distribution and severity map: Figure 7
Scientists and locations with expertise: 21

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	—
Nondormant types:	—	—

Screening procedures: none
Evaluation procedures: none

LEPTO LEAF SPOT, *Leptosphaerulina briosiana* (Poll.) Graham & Luttrell

Distribution and severity: Figure 8
Scientists and locations with expertise: 10, 11, 15, 21, and 41

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	Ranger
Nondormant types:	—	Moapa 69

Screening procedures: L and F
Evaluation procedures: May be possible on occasion in field

PHYTOPHTHORA ROOT ROT, *Phytophthora megasperma* Drechs.

Distribution and severity map: Figure 9
Scientists and locations with expertise: 1, 9, 10, 16, 25, 32, and 37

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	Agate	Saranac
Nondormant types:	MnP-D1	Sonora

Screening procedures: L and F
Evaluation procedures: L and F (page 16)

PHYMATOTRICHUM ROOT ROT, *Phymatotrichum omnivorum* (Shear) Dug.

Distribution and severity map: none
Scientists and locations with expertise: 16

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	—
Nondormant types:	—	—

Screening procedures: none
Evaluation procedures: none

RHIZOCTONIA CROWN ROT and ROOT CANKER, *Rhizoctonia solani* Kuehn

Distribution and severity map: Figure 10
Scientists and locations with expertise: 9, 10, 22, and 35

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	—
Nondormant types:	—	—

Screening procedures: L
Evaluation procedures: none

RUST, *Uromyces striatus* Schroet.

Distribution and severity map: none
Scientists and locations with expertise: 8 and 15

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	MSA-CW3AN3	Ranger
Nondormant types:	—	—

Screening procedures: L and F
Evaluation procedures: May be possible on occasion in field

SCLEROTINIA CROWN and STEM ROT, *Sclerotinia sclerotirum* (Lib.) de By. *Sclerotinia trifoliorum* Ericks

Distribution and severity map: Figure 11
Scientists and locations with expertise: 8

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	—
Nondormant types:	—	—

Screening procedures: L and F
Evaluation procedures: none

SPRING BLACKSTEM, *Phoma medicaginis* Malb. & Roum. (Syn. *Ascochyta imperfecta*)

Distribution and severity: Figure 12
Scientists and locations with expertise: 14, 21, and 41

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	Ramsey	Lahontan, Ranger
Nondormant types:	—	—

Screening procedures: L and F
Evaluation procedures: May be possible on occasion in field

STAGONOSPORA, CROWN and ROOT ROT and LEAFSPOT, *Stagonospora meliloti* (Lasch) Petr.

Distribution and severity map: none
Scientist and locations with expertise: 9

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	—
Nondormant types:	—	—

Screening procedures: none
Evaluation procedures: none

STEMPHYLIUM LEAF SPOT, *Pleospora herbarum* (Pers. ex Fr.) Rab. (*Stemphylium botryosum* Wallr.)

Distribution and severity map: Figure 13
Scientists and locations with expertise: 11, 13, 37, and 40

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	—
Nondormant types:	—	—

Screening procedures: F
Evaluation procedures: May be possible on occasion in field

SUMMER BLACK STEM, *Cercospora medicaginis* Ell. & Ev.

Distribution and severity map: Figure 14
Scientists and locations with expertise: 14, 36, and 38

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	Lahontan
Nondormant types:	—	—

Screening procedures: F
Evaluation procedures: May be possible on occasion in field

YELLOW LEAF BLOTCH, *Pseudopeziza jonesii* Nannf.

Distribution and severity map: Figure 15
Scientists and locations with expertise: 33

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	Teton	Ranger
Nondormant types:	—	—

Screening procedures:
Evaluation procedures: May be possible on occasion in field

STANDARD TESTS TO CHARACTERIZE PEST RESISTANCE IN ALFALFA VARIETIES

ARS-NC-19

ERRATA

(Add to list of authors in PREFACE — following E. L. Sorensen)

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Substitute maps

ALFALFA DWARF
Distribution and severity map: none
Scientists and locations with expertise: 9 and 37
Check varieties: *Resistant* *Susceptible*
Dormant and semidormant types: — —
Nondormant types: — —
Screening procedures: none Evaluation procedures: none

ALFALFA MOSAIC
Distribution and severity map: Figure 16
Scientists and locations with expertise: 10, 12, and 14
Check varieties: *Resistant* *Susceptible*
Dormant and semidormant types: — —
Nondormant types: — —
Screening procedures: L Evaluation procedures: none

WITCHES BROOM
Distribution and severity map: none
Scientists and locations with expertise: 29
Check varieties: *Resistant* *Susceptible*
Dormant and semidormant types: — —
Nondormant types: — —
Screening procedures: none Evaluation procedures: none

CHARACTERIZING INSECT RESISTANCE

Background

More than 100 insect species have been recorded as injurious to alfalfa according to App and Manglitz (*1*). However, in a survey of the United States, only ten alfalfa insects were identified by research and extension specialists as serious over large areas. Additional insects were identified as local problems. This report is restricted to the 10 widely distributed insect pests.

Insect pests described in this report injure the alfalfa crop in several ways. Some reduce plant yields and stands by consuming portions of the plant or by sucking plant juice. Others influence yield and quality by injecting toxins into the plant that cause stunting and distorted growth. Others attack the flowering parts or developing seed and thus, reduce seed yields. Insects also can serve as vectors of plant viruses and mycoplasmas or provide avenues of entrance for disease organisms. Insect mobility and the many types of feeding injuries they produce often makes breeding for insect resistance more difficult than breeding for some types of disease resistance. The recent identification of biotypes for some alfalfa insects further complicates breeding for insect resistance. This is especially true for the pea aphid and spotted alfalfa aphid.

Considering the complexities of insect, host-plant, and environment interactions, it is not surprising that reliable evaluation procedures are available for only the alfalfa seed chalcid, alfalfa weevil, potato leafhopper, pea aphid, and spotted alfalfa aphid. Unfortunately, only the pea aphid, spotted alfalfa aphid and alfalfa seed chalcid tests can be conducted in the laboratory or greenhouse. Most insect screening and evaluation procedures depend on natural field infestations of the target insect. This creates uncertainties about levels and uniformity of infestations among test years. It also creates the problem that damage by the target insect may be confounded with disease injury and with injury caused by other insects.

Although more difficulties appear to arise with insect resistance evaluations than with disease evaluations, the same principles should apply in evaluating varietal resistance to both types of pests. This requires that relatively uniform insect infestion levels can be developed and repeated in subsequent tests. It is a desirable practice to periodically check the identification of the test insect species, because similar appearing species may confound the results. Whenever possible, variety evaluations should be based on scoring individual plants rather than solid seeded plots. A numerical rating system, that is based on specific levels of damage or on insect population counts per plant should be used. Rating values should be consistent from one test to the next. The use

of percentage resistant plants, average severity index (ASI), or average population counts per plant, or both, should be used to statistically compare varieties with standard seed lots of resistant and susceptible check varieties.

Insect host-plant resistance can often be expressed in such specific terms as nonpreference, tolerance, and antibiosis (*41*). This may often be difficult information to obtain, but when it is available it should be used when describing variety evaluations. This could promote a better understanding of insect resistance, and it could be useful in planning pest management programs.

Summary of resistance information by insect species

Distribution and severity maps for six of the most widespread insect species in the United States appear on pages 11 and 12. A listing of scientists who can serve as resource people for problems concerned with developing host-plant resistance is included. Check varieties have been identified where sufficient information is available. The availability and type of screening and evaluation procedures (L = laboratory, F = field test) are also indicated.

ALFALFA PLANT BUG, *Adelphecoris lineolatus* (Goeze)
Distribution and severity map: none
[1]Scientists and locations with expertise: 1 and 30

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	—
Nondormant types:	—	—

Screening procedures: F Evaluation procedures: none

ALFALFA SEED CHALCID, *Bruchophagus roddi* Gussakovskii
Distribution and severity map: none
Scientists and locations with expertise: 6, 24, 26, 29, and 32

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	Lahontan	—
Nondormant types:	Lahontan	Sonora

Screening procedures: F and L Evaluation procedures: F and L (page 17)

ALFALFA WEEVIL, *Hypera postica* (Gyllenhal)
Distribution and severity map: Figure 17
Scientists and locations with expertise: 1, 2, 3, 5, 7, 31, 34, 36, 39, and 42

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	Team, Arc	Ranger, Vernal
Nondormant types:	—	—

Screening procedures: F and L Evaluation procedures: F (page 18)

EGYPTIAN ALFALFA WEEVIL, *Hypera brunneipennis* Boheman
Distribution and severity: Figure 18
Scientists and locations with expertise: 6, 22, 26, 32, and 39

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	—
Nondormant types:	—	—

Screening procedures: F Evaluation procedures: none

LYGUS SP.
Distribution and severity map: none
Scientists and locations with expertise: 6, 24, 26, 29, and 32

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	—
Nondormant types:	—	—

Screening procedures: F Evaluation procedures: none

MEADOW SPITTLEBUG, *Philaenus spumarius* (L.)
Distribution and severity map: Figure 19
Scientists and locations with expertise: 34 and 42

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	Culver	Ranger
Nondormant types:	—	—

Screening procedures: L Evaluation procedures: none

[1]Key for names of scientists that can serve as resource personnel listed on page 8

PEA APHID, *Acyrthosiphon pisum* (Harris)
Distribution and severity map: Figure 20
Scientists and locations with expertise: 6, 17, 18, 19, 20, 23, 24, 28, 32, and 36

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	Kanza	Ranger
Nondormant types:	T3-12	Caliverde, Moapa 69

Screening procedures: L Evaluation procedures: L (page 18)

POTATO LEAFHOPPER, *Empoasca fabae* (Harris)
Distribution and severity map: Figure 21
Scientists and locations with expertise: 4, 7, 15, 17, 19, 20, 23, 31, 34, 36, and 42

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	MSA-CW3AN3	Ranger
Nondormant types:	—	—

Screening procedures: F Evaluation procedures: F (page 19)

SPOTTED ALFALFA APHID, *Therioaphis maculata* (Buckton)
Distribution and severity map: Figure 22
Scientists and locations with expertise: 6, 17, 18, 19, 20, 22, 23, 24, 28, 32, and 36

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	Kanza	Ranger, Team
Nondormant types:	Mesa-Sirsa	Caliverde

Screening procedures: L Evaluation procedures: L (page 19)

WESTERN POTATO LEAFHOPPER, *Empoasca abrupta* Delong
Distribution and severity map: none
Scientists and locations with expertise: 6, 26, and 32

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	—
Nondormant types:	—	—

Screening procedures: none Evaluation procedures: none

CHARACTERIZING NEMATODE RESISTANCE

Background

Limited knowledge of the damage caused by many of the nematodes associated with alfalfa is available according to Graham, Kreitlow, and Faulkner (*15*). This is due in part to lack of research on alfalfa nematodes and in part to the lack of distinctive above ground symptoms caused by nematode injury. Presently, the stem nematode and two species of root-knot nematode are the primary parasitic nematode species known on alfalfa. Resistant varieties or germplasm have been developed to all three species.

Summary of resistance information by nematode species

Distribution and severity maps were prepared for the three primary parasitic nematode species on alfalfa. Included for each species is a list of scientists working on that species. They can serve as resource people for problems concerned with developing nematode resistant varieties. Check varieties and standardized screening procedures have been established for the three types of nematode resistance. Availability and type of screening and evaluation procedures (L = laboratory, F = field tests) have also been indicated.

STEM NEMATODE, *Ditylenchus dipsaci* (Kuhn) Filipjev
Distribution and severity map: Figure 23
[1]Scientists and locations with expertise: 8, 18, and 28

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	Lahontan	Ranger
Nondormant types:	Caliverde 65	Moapa 69

Screening procedures: L Evaluation procedures: L (page 20)

NORTHERN ROOT-KNOT NEMATODE, *Meloidogyne hapla* Chitwood
Distribution and severity map: Figure 24
Scientists and locations with expertise: 8, 18, and 28

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	Nev. Syn. XX	Lahontan
Nondormant types:	Nev. Syn. YY	Mesa-Sirsa

Screening procedures: L Evaluation procedures: L (page 20)

[1]Key for names of scientists that can serve as resource personnel listed on page 8.

SOUTHERN ROOT-KNOT NEMATODE, *Meloidogyne incognita* Chitwood and *Meloidogyne javanica* (Treub) Chitwood

Distribution and severity map: Figure 24

Scientists and locations with expertise: 8, 18, and 28

Check varieties:	*Resistant*	*Susceptible*
Dormant and semidormant types:	—	Lahontan
Nondormant types:	Moapa 69	Caliverde 65

Screening procedures: L Evaluation procedures: L (page 20)

KEY TO NAMES OF RESOURCE SCIENTISTS

This list includes scientists familiar with various phases of alfalfa pest resistance research from State agricultural experiment stations and the U.S. Department of Agriculture (USDA). This is not a complete list of scientists working on pest resistance. It is primarily intended to be a list of resource personnel who are knowledgeable about pest resistance evaluation procedures.

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14. E. W. Hanson, Dept. of Plant Pathology, Univ. of Wisc., Madison, Wis. 53706
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16. R. B. Hine, Dept. of Plant Pathology, Univ. of Ariz., Tucson, Ariz. 85721
17. E. Horber, Entomology Dept., Kansas State Univ., Manhattan, Kans. 66506
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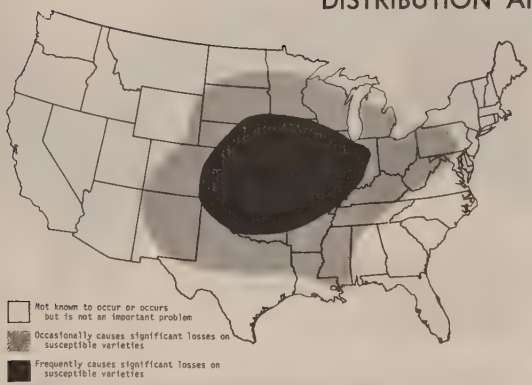
Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 1.—Bacterial leaf spot, *Xanthomonas alfalfae* (Riker, Jones & Davis) Dows, distribution and severity in alfalfa.

Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 2.—Bacterial wilt, *Corynebacterium insidiosum* (McCull.) H. L. Jens, distribution and severity in alfalfa.

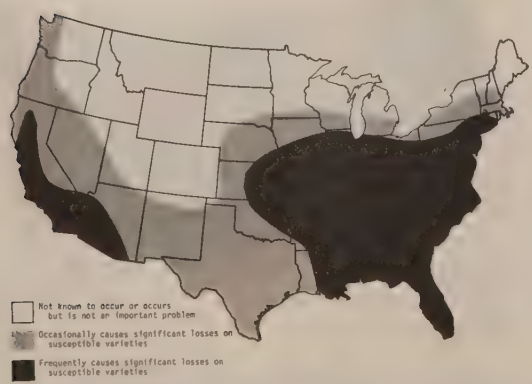
Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 3.—Anthracnose, *Collectotrichum trifolii* Bain & Essary, distribution and severity in alfalfa.

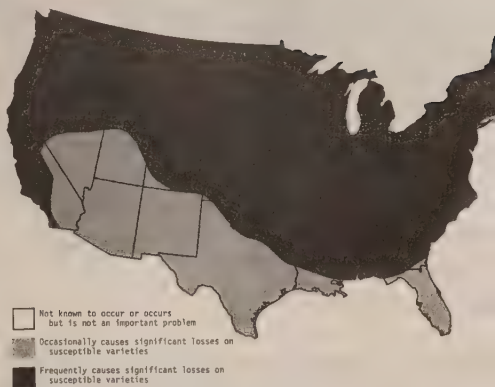
Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 4.—Common leaf spot, *Pseudopeziza medica* (Lib.) Sacc., distribution and severity in alfalfa.

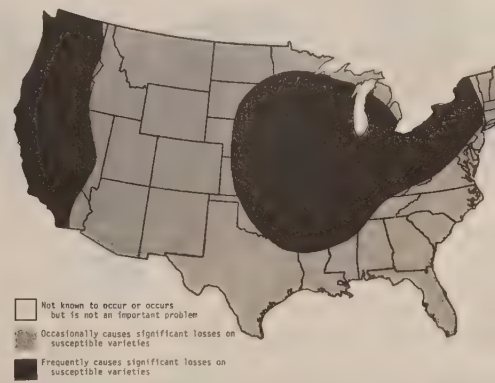
Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 5.—Downy mildew, *Peronospora trifoliorun* By., distribution and severity in alfalfa.

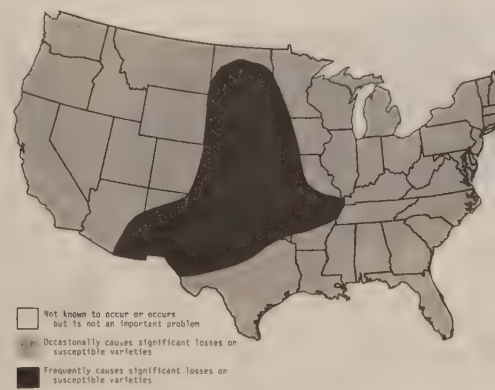
Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 6.—Fusarium wilt, *Fusarium oxysporum* Schl f. *medicaginis* (Weimer) Snyd. & Hans, distribu and severity in alfalfa.

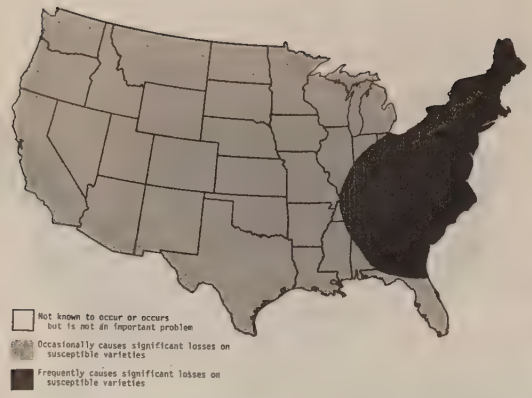
Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 7.—Fusarium root and crown rot, *Fusarium solani* (Mart.) Appel & Wr., *F. roseum* LK, distribution and severity in alfalfa.

Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 8.—Lepto leafspot, *Leptosphaerulina briosiana* (Poll.) Graham & Luttrell, distribution and severity in alfalfa.

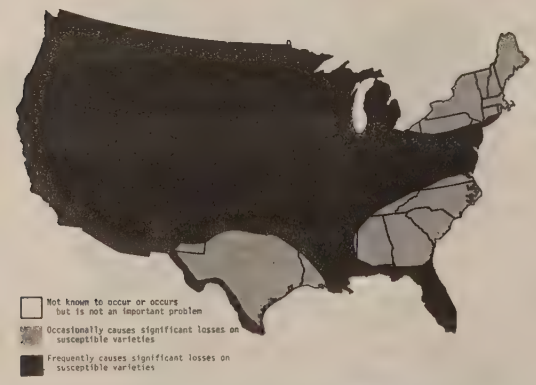
Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 9.—Phytophthora root rot, *Phytophthora megasperma* Drechs, distribution and severity in alfalfa (confined to wet soils).

Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 10.—Rhizoctonia crown rot and root canker, *Rhizoctonia solani* Kuehn, distribution and severity in alfalfa (root canker primarily in California, Nevada, Arizona, and New Mexico).

Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 11.—Sclerotinia crown and stem rot, *Sclerotinia sclerotiorum* (Lib.) de By. and *S. trifoliorum* Ericks, distribution and severity in alfalfa.

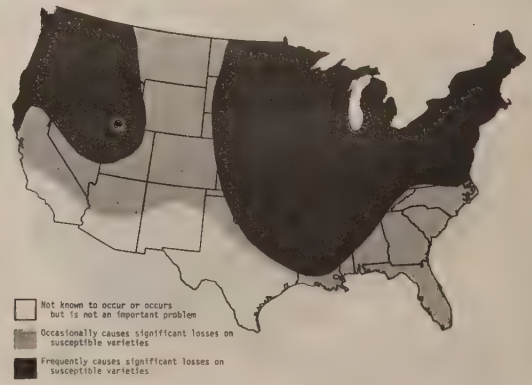
Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 12.—Spring blackstem, *Phoma medicaginis* Malb. & Roum. (*Ascochyta imperfecta*), distribution and severity in alfalfa.

Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 13.—Stemphylium leafspot, *Pleospora herbarum* (Pers. ex. Fr.) Rab. (*Stemphylium botryosum* Wallr.), distribution and severity in alfalfa.

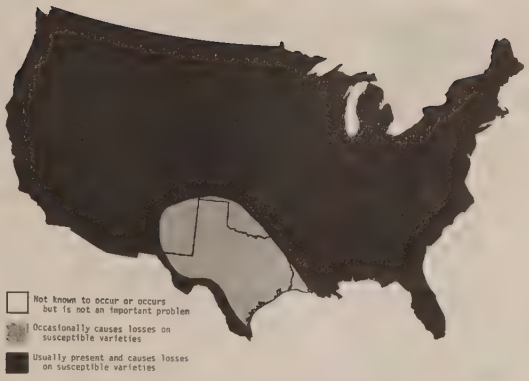
Not known to occur or occurs but is not an important problem
Occasionally causes losses on susceptible varieties
Usually present and causes losses on susceptible varieties

FIGURE 16.—Alfalfa mosaic virus, distribution and severity in alfalfa.

Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 14.—Summer black stem, *Cercospora medicaginis* Ell. & Ev., distribution and severity in alfalfa.

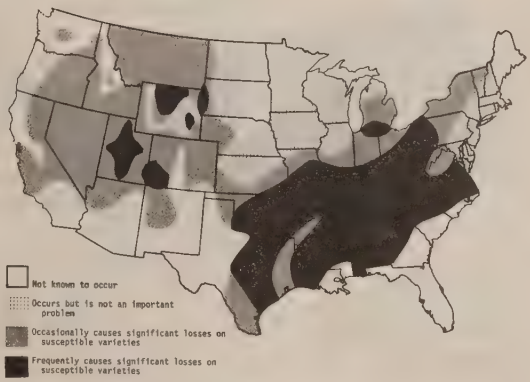
Not known to occur
Occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 17.—Alfalfa weevil, *Hypera postica,* distribution and severity on alfalfa (populations still in transition).

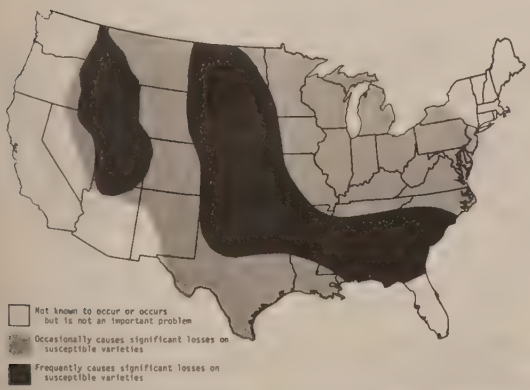
Not known to occur or occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 15.—Yellow leaf blotch, *Pseudopeziza jonesii* Nannf., distribution and severity in alfalfa.

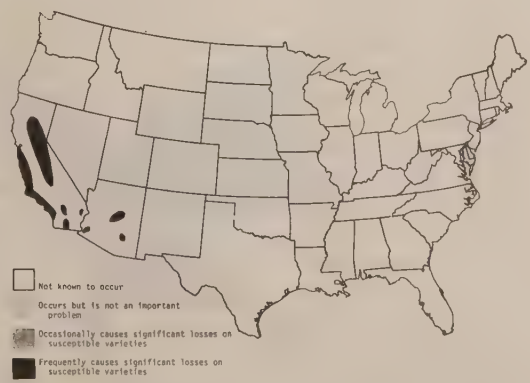
Not known to occur
Occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 18.—Egyptian alfalfa weevil, *Hypera brunneipennis* Boheman, distribution and severity on alfalfa.

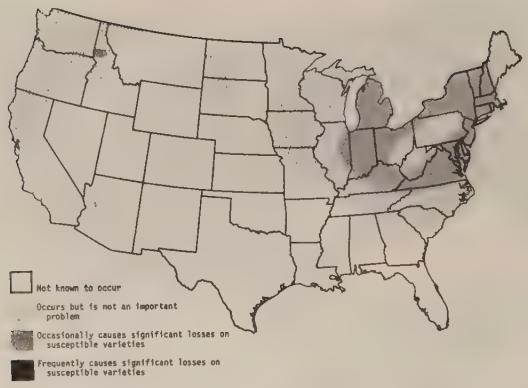
Not known to occur
Occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 19.—Meadow spittlebug, *Philaenus spumarius* (Linnaeus), distribution and severity on alfalfa.

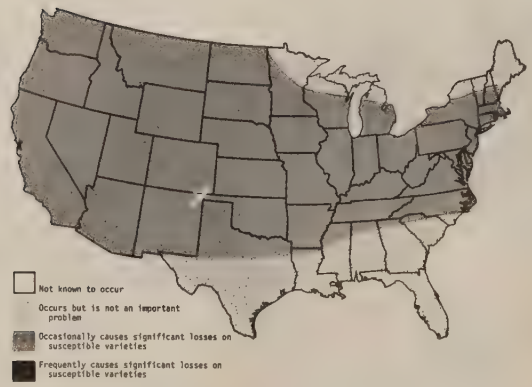
Not known to occur
Occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 20.—Pea aphid. *Acyrthosiphon pisum* (Harris), distribution and severity on alfalfa.

Not known to occur
Occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 21.—Potato leafhopper, *Empoasca fabae* (Harris), distribution and severity on alfalfa.

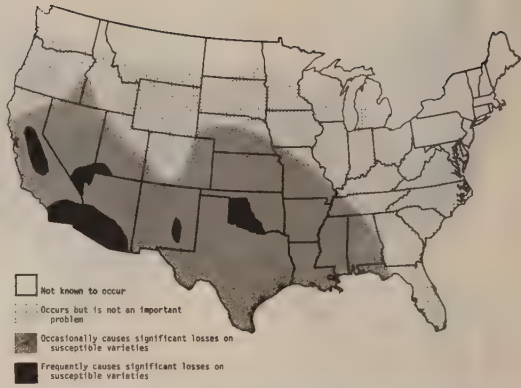
Not known to occur
Occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 22.—Spotted alfalfa aphid, *Therioaphis maculat* (Buckton), distribution and severity on alfalfa.

Not known to occur
Occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 23.—Stem nematode, *Ditylenchus dipsaci* ([Kuhn] Filipjev), distribution and severity on alfalfa.

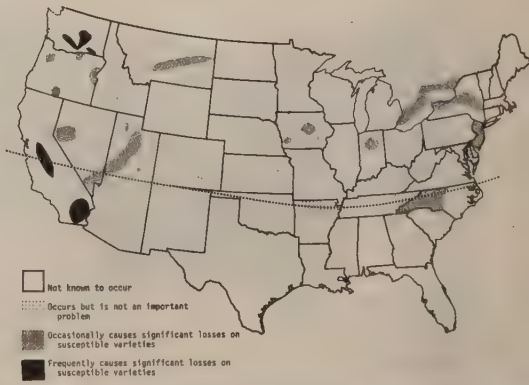
Not known to occur
Occurs but is not an important problem
Occasionally causes significant losses on susceptible varieties
Frequently causes significant losses on susceptible varieties

FIGURE 24.—Root-knot nematode, *Meloidogyne* sp., dis tribution and severity on alfalfa (southern specie below line, northern species above line).

ESTABLISHED VARIETY EVALUATION PROCEDURES

Few examples of continuous long term pest resistance tests are available for alfalfa. The oldest program in the United States is the 25-year-old bacterial wilt test conducted at the University of Minnesota. Many of the evaluation procedures for other types of pest resistance are more recent or have been used only sporadically. For these reasons, to refer to these procedures as standardized evaluation tests would be presumptuous of this committee. Nevertheless, many of the tests can be used for variety evaluations.

Established evaluation procedures were available for 13 of the 35 major alfalfa pests listed in this publication. Scientists experienced with the tests were asked to prepare brief summaries of their methods. These summaries should provide a useful set of guidelines for future evaluations.

Evaluating bacterial wilt resistance

Methods used at University of Minnesota:

The most reliable method of evaluating alfalfa varieties for bacterial wilt resistance has been the "bare-root soak" method of inoculating and then growing the plants from 3 to 4 months in the field. This method was developed by Cormack, Peake, and Downey (*8*) and modified by Elling and Frosheiser (*11*).

Bacterial wilt-infected roots, free of other diseases are selected from previous field tests as inoculum. The crown is removed, then the roots are washed, ground, packed in plastic bags, and stored at −18°C (*30*). Bacteria will remain virulent several years in continuously frozen material. About one-half hour before inoculation a bacterial suspension is prepared by soaking the infected root material (50 g/liter) in tap water. The ground root material is left in the water throughout the inoculation period.

Seedling plants can be grown in any medium before being inoculated. At Minnesota seedlings are grown for at least 8 weeks in sand. Plants are inoculated with rhizobium, fertilized, and watered to promote maximum growth. Using sand makes pulling the plants and washing the roots easy. Plants are individually separated and small ones are discarded. About 75 plants per replication are tied in a bundle. Three replications are grown of each entry.

Roots must not dry out before inoculation. Inoculation is accomplished by immersing bundles of plants in the bacterial suspension for 20 to 30 minutes. The bundles of plants are then removed, the tops cut off about 5 cm above the crown, and the roots trimmed to about 10 cm. Severa' bundles are wrapped together in paper toweling containing a small amount of wet vermiculate. This helps to keep the roots moist until transplanting. The inoculated plants wrapped in this manner and placed in 1 to 2 cm of water can be stored for several days under refrigeration before transplanting.

Inoculated plants are transplanted to the field in early June with a mechanical transplanter. Plants are spaced 15 to 20 cm apart within the row with 1 m between rows. The field is cultivated and irrigated to maintain vigorous plant growth throughout the summer. Harmful insects may need to be controlled. Dead plants in the very susceptible varieties are removed and recorded during August.

About three months after transplanting (September 10 to 15) the plants are undercut about 15 cm deep. The plants are pulled, and the tap roots sectioned and examined for internal discoloration. Ratings are based on a 0 to 5 scale. The classes are: 0 = sectioned surface is clean and white, 1 = very small spots of yellow-brown discoloration are visible in root cortex, 2 = discoloration is more evident with up to one-third of cortex affected, 3 = nearly complete cortex discolored but the stele is relatively white, 4 = discoloration extends throughout the root but plant is still alive, and 5 = root is severely rotted with plant dead or dying. Classes 0 and 1 are considered resistant.

Level of bacterial wilt resistance among varieties within a test can be expressed as percentages of resistant plants or as Average Severity Index (ASI). The ASI score is more precise than percentage of resistant plants and is useful in comparing relatively small differences in degree of resistance in a single test (*2*). However, percentage resistant plants is most useful in comparing varieties evaluated in different test years. That is done by expressing the data from each test as percentage of a standard resistant check variety (Vernal). The data then are adjusted according to a long-time average (table 1). Nearly all varieties grown in the United States have been evaluated at the University of Minnesota with these procedures. Data were reported in the 1970 Central Alfalfa Improvement Conference Report.

TABLE 1.—*Percent of bacterial wilt resistant plants of alfalfa varieties compared with Vernal*

Variety	Number of tests	Average of Vernal per test	Resistant plants per variety[1]
		Percent	*Percent*
Agate	2	154.0	73
Iroquois	2	145.3	69
Narragansett	13	1.5	1
Ramsey	4	89.6	42
Ranger	14	35.0	17
Vernal	14	100.0	47

[1]Calculated by multiplying percent of Vernal by 47.3 (Average percent resistant plants in Vernal from 14 test years).

By F. I. Frosheiser and D. K. Barnes

Evaluating anthracnose resistance

Methods used at USDA Agriculture Research Center, Beltsville, Md.:

Alfalfa seedlings and mature plants react similarly when infected by *Collectotrichum trifolii.* This simplifies the evaluations of resistance. Seedling evaluation procedures were first described by Ostazeski, Barnes, and Hanson (*39*). The procedures have since been refined and have been used to develop resistant populations (*9*).

The inoculum source is obtained from isolates collected periodically from infected plants in the field. These can be increased on several culture media including potato dextrose agar, V-8 juice agar, and on sterilized bean leaves floated on V-8 juice broth. When agar media are used for increasing inoculum, maximum sporulation is obtained from plates seeded with fresh spore suspensions. Most isolates will grow and sporulate, but alternating on-off cycles of fluorescent light during incubation can enhance sporulation. If plate cultures are to be used as sources of inoculum, growth from about 15 (9 cm) petri plates should be scraped from the surface of the agar and should be blended into 400 ml of water. The remaining agar from five to eight plates is also blended into the mixture and acts as a sticker. The final volume is brought up to 750 ml. About 80 ml of blended inoculum is sprayed on a 30 by 60 cm flat of seedlings.

A dry inoculum method has also been developed (*6*). Diseased plants from susceptible varieties grown under controlled conditions are dried and ground. This inoculum is applied to seedlings as dust at a rate of 10 g per flat. Dry inoculum can be stored at least 10 months at −20°C without loss of virulence (*40*). Dry inoculum should not be collected from field epiphytotics because it probably contains other pathogens, especially *Rhizoctonia.*

Seedlings are grown for inoculation in steam-sterilized wooden flats, filled to within 2 cm of the top with steamed soil or Jiffy mix[2] and covered with 0.6 cm of sand, upon which seed is planted in rows. Plant spacing is 3.8 cm between rows and 2.0 cm within rows. Seedlings are grown, inoculated and scored for disease reaction in a growth chamber at 23°C, with 16-hour daylength, and 17.2 klux light intensity.

About 18 days after seeding, polyethylene sheeting is used to form a moist chamber over the plants. Two humidifiers (Model 42, Standard Engineering Co., Pawtucket, R.I.) operating two consecutive minutes out of every five during daylight hours, provide a suitable amount of mist. During dark hours humidifiers operate only during every other hour. Inoculum is applied when the foliage is first covered with water droplets. Twenty-four hours after the first inoculation, a second is applied at the same rate. Inoculated seedlings are held in the moist chamber 72 hours.

Plants are scored about 2 weeks after inoculation. Scoring is from 1 (resistant) to 5 (dead) and corresponds to lesion type (*39*). Type 1 stems have no lesions or only small water-soaked spots. Type 2 is a long, narrow lesion with few, if any, acervuli. Type 3 lesions are long, wide, not girdling, with acervuli usually present. Type 4 lesions are large, coalescing and sporulating, which eventually girdle and kill the stems. In variety evaluations Types 1 and 2 can be classified as resistant. Percentage resistant plants can be compared among varieties by relating each variety to the standard resistant check. ASI can also be used to compare varieties.

By S. A. Ostazeski

Evaluating common leafspot resistance

Laboratory and greenhouse methods used at the U.S. Regional Pasture Research Laboratory, University Park, Pa.:

Cultures of the fungus can be stored under refrigeration on oatmeal agar (36 g of Difco oatmeal agar preparation and 7 g of agar per liter

[2]An equal mixture of peat moss and vermiculite distributed by Jiffy Products of America, West Chicago, Ill.

of distilled water). These cultures are flooded with distilled water, and the surface crust of apothecia is fragmented to increase the inoculum. Approximately 1 ml of the suspension is evenly distributed over the surface of oatmeal agar in a petri dish. About 50 plates are poured per liter of agar, and an increase ratio of about 1:10 can be expected. Usually two increases are required to obtain enough culture plates for an inoculation. Aseptic conditions are maintained throughout the increases. Cultures are incubated in the dark at 20°C for 21 days. At this time a sample plate is inverted over a microscope slide from 1 to 2 hours. The slide is examined at 100X magnification to ensure that the cultures are sporulating.

Seedlings for inoculation are grown in metal flats filled with either soil or Jiffy mix or a 1:1 mixture of the two. We plant on about 4 cm centers and inoculate the seedlings when they have 7 to 10 fully expanded trifoliate leaves. The plant takes approximately 30 days to reach that stage in the greenhouse.

Plants are moved to an inoculation-incubation chamber that is maintained at 20°C in the dark with saturated relative humidity. Sporulating cultures are suspended about 20 cm above the tops of the plants. The dishes are spaced on about 15 cm centers and rearranged periodically to ensure more uniform coverage. The spores are small enough that some lateral dispersion occurs. Cultures are allowed to deposit spores for about 8 hours before the plant surfaces are sprayed with a fine mist. The mist is terminated just before runoff. Cultures are left to deposit spores on the plants for 48 hours. Plants are retained in the moist chamber an additional 24 hours and are allowed to dry slowly before being moved back into the greenhouse.

Because the plants have been in the dark at a high humidity, one should not immediately expose them to full sunlight. First move them into subdued light and then during late afternoon and early evening move them into the greenhouse. At temperatures of 20° to 26°C symptoms develop in about 3 weeks after inoculation. Resistance is determined by the size of lesions, the presence or absence of a central fruiting structure (apothecium), and leaf chlorosis and defoliation. We recommend a 1 to 5 scale for scoring disease severity. The classes are 1 = no spots; 2 = very small black spots without apothecia; 3 = small black spots with small apothecia; 4 = large black or brownish spots with large apothecia; and 5 = large black or brownish spots with large apothecia accompanied by chlorosis and defoliation.

Plants rated 1 and 2 are considered resistant. Leaves that are covered with class 2 spots may not be truly resistant. When leaves have been heavily infected, spots may not develop normally. Usually, heavily infected leaves of susceptible plants will turn yellow and drop off. The chlorosis that is typical of class 5 susceptibility on heavily infected susceptible leaves may be more striking in the field than in the greenhouse. A susceptible check is useful in estimating the frequency of escapes (plants rated in class 1). A resistant check is used to compare results between tests.

By K. T. Leath and R. R. Hill, Jr.

Field methods used at University of Minnesota:

Plants spaced about 30 cm apart in the row and 60 cm between rows allows one to examine individual plants with space to maneuver between rows. Wider spacing may result in less infection. Alternate rows of a susceptible variety may be useful in increasing disease potential, but this usually does not appear to be necessary. Natural epiphytotics of common leafspot usually occur in Minnesota and adjacent states during late summer or early fall. These epiphytotics are often relatively pure and do not contain other diseases, especially in first year plantings. Plantings made in early May should be clipped once in mid-July and then allowed to mature until an epiphytotic develops.

Each plant should be examined individually and scored on a 1 to 5 scale. The same scale as suggested for laboratory testing can be used in field evaluations. In 1971 Minnesota field tests, the varieties DuPuits, Cayuga, and Atlantic were scored for common leafspot resistance along with 107 other varieties. Percentages of resistant plants for the three varieties were 76, 40, and 14, compared with 80, 32, and 20 percent reported by Graham and others (*14*) in greenhouse tests. Both laboratory and field tests appear suitable to evaluate varieties.

By F. I. Frosheiser

Evaluating downy mildew resistance

Laboratory methods used at Kansas State University:

Initial inoculum is collected from diseased plants in the field. Physiological races occur in

the fungus so a diverse collection of diseased plants is recommended. Sporangia from the field often germinate poorly, so it is advisable to build up inoculum on seedlings ahead of evaluation tests. Thickly seeded pots of susceptible alfalfa can be included in each test to provide inoculum for the next test. A few sporangia on diseased seedlings harvested and stored at −20°C will remain viable for a few weeks.

Plants are grown and inoculated in growth chambers maintained at 20°C and 1000 FC of continuous fluorescent light, except for designated dark periods. A schedule was developed that required little attention during weekends:

Day 1 (Friday)	Plant seeds 1.3 cm deep in rows in flats of sand. Rows are spaced 2.5 cm apart and seeds spaced 2 to 3 cm within rows.
Day 5 (Tuesday, p.m.)	Inoculate, cover plants, and turn off lights at least 12 hours (overnight).
Day 6 (Wednesday, a.m.)	Uncover plants, turn on lights, and rogue any plants emerged since inoculation.
Day 7-10	Rogue any newly emerged plants.
Day 11 (Monday, p.m.)	Cover plants, turn off light for at least 12 hours to induce sporulation.Do not water plants just before covering because the fungus will not sporulate in free water.
Day 12 (Tuesday)	Evaluate symptoms on plants.

On the fifth day inoculations are made by placing diseased plant tissue in a covered jar with deionized water. The jar is shaken vigorously to dislodge sporangia. The spore suspension is strained through a tea strainer to remove plant debris. A minimum of 25,000 viable sporangia per millimeter are needed for uniform infection. The inoculum is sprayed onto the plants until a droplet forms between the cotyledons. To prevent the plants from drying off for at least 12 hours, the flats are placed in trays and airtight covers are inverted over the flats. The same covering procedure is used on day 11 to induce sporulation.

Variety evaluation is based on the percentage of resistant plants, those without symptoms. Susceptible and resistant check varieties are included in each flat to provide a basis for comparing varieties evaluated in different tests. A minimum of four, 40 plant replications are suggested.

By D. L. Stuteville

Field methods used at University of Minnesota:

Spaced plants in the field can also be used to evaluate levels of downy mildew resistance in alfalfa varieties (*42*). Field epiphytotics are usually observed on succulent vegetative growth during early spring or late fall.

In the field it is possible to classify individual plants according to degrees of resistance. A four class scale has been used at Minnesota and Utah (Class 1 = no symptoms, Class 2 = small usually nonsporulating lesions on one or two leaves, 3 = sporulating lesions on 10 to 25 percent of the leaves, and 4 = general infection over the entire plant). Plants classified as 1 or 2 are considered resistant. Varieties can be statistically compared with susceptible and resistant check varieties by either ASI or percentage resistant plants.

By D. K. Barnes

Evaluating phytophthora root rot resistance

Field methods used at University of Minnesota:

Select a level area with relatively heavy soil. If the soil is not naturally infested with *Phytophthora megasperma*, infested soil may be spread over the area and incorporated into the upper 15 cm. Infested soil can be from naturally infested fields or from greenhouse experiments.

Seed is planted in early May at about 75 viable seeds per 1.6 m of row, in rows about 30 cm apart. A minimum of three replications per entry is suggested. A stand count is taken approximately 2 weeks after planting, while the seedlings are in the unifoliolate stage. After the seedlings are about 4 weeks old, rainfall must be supplemented with irrigation to keep the surface soil continuously at or near saturation for two to three weeks. After the first 2 to 3 week wet period, the soil is allowed to dry. This allows time to take notes and cultivate. If the disease is severe, the plants may be evaluated at this time. However, one, and preferably two, additional 3-week wet periods reduces the frequency of escapes. This provides a more accurate evaluation of the degree of root rot on each plant because the roots are larger. Plants are dug and scored during the first 2 weeks of September.

When plants are dug, as much of the root system as possible should be retained. At least 30 cm of tap root is needed. All the plants from a row are dug and tied in a bundle. The roots are first soaked in a tub of water and then are washed

with water under pressure. Plants are individually classified for degree of root rot. We prefer to do all classification under a uniform light source in the greenhouse, rather than in the field.

A 1 to 6 classification scale described by Frosheiser and Barnes (*13*) is used: 1 = roots are clean with no lesions and many small rootlets are present on the tap root; 2 = only very small lesions (2 mm) are present on tap root, tap roots usually lack branch roots, and most lesions occur at site where branch root had started growth; 3 = one or more large lesions on tap root, but none girdling the tap root, the tips of a few larger branch roots are rotted off; 4 = extensive root lesions with the tap roots usually rotted off 15 cm or more below the crown; 5 = tap root almost completely destroyed, but plant alive; and 6 = plants dead (calculated as loss in stand count from 2 weeks after seeding to mid-September). Plants rated 1 and 2 are considered resistant. All tests should contain a resistant check and a susceptible check.

Level of resistance among varieties within a test can be expressed as percentage of resistant plants or as ASI. Percentage of resistant plants can be used to compare varieties in different tests. This procedure was previously described in the bacterial wilt evaluation procedures and consists of expressing the data from each test as percent of the resistant check variety, Agate, then adjusting the data according to Agate's longtime average of 55 percent resistant plants. Most varieties grown in the United States have been tested at the University of Minnesota. Data from these tests were included in the 1971 Central Alfalfa Improvement Conference Report.

Greenhouse methods used at University of Minnesota:

Greenhouse tests are conducted in 20-cm deep, water-tight containers filled with fine, steamed sand (*13*). The sand is initially infested by using laboratory cultures. The fungus is grown on V-8 juice agar (200 ml V-8 juice, 20 g agar, 1 g $CaCO_3$ in 800 ml water) in 9 cm petri dishes. Two-week-old cultures are blended in water and added to the sand at one petri dish to 500 cm^2 surface area. The inoculum is mixed with the sand.

Seed is planted directly into the sand. The seedlings are watered sparingly until they are well established (about 4 weeks). Then the drainholes in the tanks are plugged and enough water is added daily to raise the water level to the sand surface. Sand temperatures of 20° to 24°C are maintained throughout this period because it is optimum for *Phytophthora* root rot. After about 4 weeks of continual flooding the plants can be evaluated for root rot as described in field evaluations. Resistant and susceptible check varieties should be included in each test for comparisons.

Except in unusual cases no additional inoculum needs to be added to the sand for repeated testing. Field and greenhouse evaluations were correlated (r = 0.99 and 0.95) in two tests (*13*). However, we think field tests give a more accurate evaluation of varieties because the roots are larger and easier to score and more plants can be observed. Nevertheless, greenhouse procedures are useful for screening and are being used successfully by a number of plant breeders (*16*).

By F. I. Frosheiser and D. K. Barnes

Evaluating alfalfa seed chalcid resistance

Methods used at Tucson, Ariz.:

Evaluations of seed chalcid resistance can be conducted either in the field or in the greenhouse. Field evaluation of varieties is begun by establishing plants in rows spaced 1 m apart, with 50 to 60 cm between plants within rows. Six replications are usually used. Fields are managed, so that plants are setting seed during August when alfalfa seed chalcids reach maximum populations. Samples of 25 racemes, with fully developed pods, are taken from each plant and are placed in a pint sized ice cream carton. Adult chalcids are allowed to emerge. Statistical comparisons of varieties are made on the basis of the mean number of adults per 25 racemes per plant (*36*).

Greenhouse evaluations are made by caging 10 adult chalcids on a raceme. From 6 to 8 racemes are caged per plant. The adult chalcids are reared from infested seed screenings held in storage at 10°C. After 2 weeks the caged racemes are removed and placed in rearing cartons. The number of emerged adult chalcids are counted after 2 weeks (*34*). As with field procedures varieties are compared by mean number of emerged adults per plant. However, varieties are also rated according to percent resistant plants. Resistant plants are those that average no more than one adult per raceme. All field and greenhouse tests should contain resistant and susceptible check varieties.

By M. W. Nielson

Evaluating alfalfa weevil feeding resistance

Methods used at USDA Agricultural Research Center, Beltsville, Md. and at North Carolina State University:

Methods are available for laboratory selection of germplasm with larval antibiosis and nonpreference to adult feeding and oviposition. However, present levels of these mechanisms of resistance are too low and interactions with field observations are too high for variety evaluation. In field tests, weevil resistant selections have been consistently separated on the basis of larval-feeding damage. Thus, field testing in areas with natural weevil infestations is the only practical method for evaluating varieties for weevil resistance. This approach also appears justified because tolerance to larval feeding is the principle mechanism of resistance (*3*). Tolerance may be related to fall and spring growth characteristics plus the plant response to weevil injury.

Test entries should be seeded in broadcast or drill-row plots at least 4.5 m^2. Spaced plants have not been well adapted to alfalfa weevil evaluations. At least four replications of each entry, including resistant and susceptible check varieties, should be included in each trial. Larval feeding damage is evaluated by estimating the percentage of leaves eaten (percent defoliation) on the basis of total leaves available. Percentage of defoliation is estimated for the entire plot. Data for each entry should then be converted and presented as percent defoliated and compared with the resistant check, using a base of 100 for the check. An example of this procedure is shown in tables 2 and 3. The data on percent defoliation are shown in table 2 and the converted data are shown in table 3. More than one evaluation date is necessary to determine the performance of most entries accurately.

TABLE 2.—*Alfalfa weevil larval feeding damage on alfalfa varieties at Raleigh, N.C. 1970*[1]

Variety	Defoliation		
	April 22	May 1	May 6
	Percent	*Percent*	*Percent*
MSHp6F	30.0	26.7	46.7
Saranac	52.5	63.3	75.5
Atlantic	63.3	71.7	78.3
Cherokee	66.7	78.3	78.3
Team (check)	28.3	36.7	51.7

[1]Taken from table 10, p. 25, 1969-70 Annual Report Alfalfa Breeding Investigations, Raleigh, N.C. Data for May 11 omitted.

TABLE 3.—*Alfalfa varieties classified for tolerance to alfalfa weevil larval feeding by percent defoliation in relation to the check variety, Team. Raleigh, N.C. 1970*

Variety	Defoliation of Team[1] by date			
	April 22	May 1	May 6	Average
	Percent	*Percent*	*Percent*	*Percent*
MSHp6F	106	73	90	90
Saranac	186	172	146	168
Atlantic	224	195	151	190
Cherokee	236	213	151	197
Percent defoliation of Team	28.3	36.7	51.7	38.9

[1]Team = 100.

By R. H. Ratcliffe

Experience indicates that conditions which influence plant growth and insect infestation levels cannot be controlled in the field. Therefore, obtain at least 2 years data on each set of plots. It might be possible to reduce the variation because of differences in alfalfa weevil population levels (both seasonal and between locations) if tests were located only in areas of known high weevil infestation. However, that would necessitate testing many lines or varieties outside of their area of development and adaptation, which could alter their tolerance to weevil feeding. For this reason it is difficult to recommend a specific test location for alfalfa weevil testing. Entomologists and plant breeders involved in developing resistant varieties will need to determine appropriate test locations based on weevil pressures and variety adaptation.

Evaluating pea aphid resistance

Methods used at Kansas State University:

Several methods and resistance criteria have been effective for evaluating resistance of alfalfa varieties to the pea aphid (*7*, *22*, *38*, and *46*). Plant resistance is similar at various stages of growth in both laboratory and field. We utilize a seedling test under controlled conditions.

Numbers of aphids needed to evaluate resistance can be increased in the greenhouse on succulent susceptible alfalfa or on broadbeans, *Vicia faba* L. (*Faba vulgaris* Moench). However, broadbeans should be used with caution because aphids

may become conditioned to them, which could, in time, influence their reaction on alfalfa.

Evaluation tests are conducted in either a growth chamber or greenhouse. Greenhouse tests are most practical, but aphids tend to congregate at the sides or ends of flats as temperatures and light intensities change. Tests conducted in growth chambers at 20°C, 55 to 65 percent RH, with a 16-hr photoperiod at 1250 FC of light are more precise than greenhouse tests.

Seedlings are established in rows in greenhouse flats. A minimum of four, 50-plant replications is recommended. Scarified seeds are planted on steam-sterilized soil and covered with 1.3 cm of sand. Flats are placed in aluminum trays for subirrigation. Seedlings in the early cotyledon stage are infested by shaking large numbers of aphids over the seedlings. Additional aphids are added as needed to maintain a high population. Plants and insects are enclosed with 25 cm high glass cages with plastic screen tops. When most plants of the susceptible check variety are dead, the test is terminated. Surviving seedlings in each row are counted. Resistance is expressed as percent seedling survival and is compared with that of a resistant check variety. It should be noted that in some southwestern states, large field cages have been used successfully for aphid evaluations.

By E. L. Sorensen

Evaluating potato leafhopper yellowing resistance

Methods used at University of Nebraska:

Attempts have been made to develop seedling tests to evaluate potato leafhopper resistance in alfalfa (*31*, *33*, *48* and *49*). However, the mechanisms of resistance are not well defined. Resistance to leafhopper yellowing under natural field infestations has been the most useful trait found for evaluating varieties.

We transplant 6- to 8-week-old seedlings from the greenhouse to the field in late April or early May. A minimum of four replications of 25 plants each is recommended. Plots consist of a single row with plants spaced about 30 cm apart in rows. We have successfully varied spacing between rows from about 30 to 90 cm. Plantings are kept weed free, and insecticides are avoided.

The time between planting and scoring for yellowing (or reddening depending on plant genotype) is critical. Harvest intervals are extended about 10 to 20 days beyond the normal 35-day cutting interval. Usually plants are scored twice during the growing season. A single score, however, may be sufficient if the plants have grown long enough to have been exposed to a severe infestation of leafhoppers nymphs (*29*). Infestation levels can be compared among studies in such units as nymphs per grams of dry matter (*28*).

Individual plants are visually scored for leafhopper yellowing using the 1 to 9 scale described in the 19th Alfalfa Improvement Conference Report (CR-54-56: 63, 1964). A score of 1 = up to 10 percent of leaves show yellowing, 2 = between 10 and 20 percent of leaves yellow, 3 = between 20 and 30 percent of leaves yellow, 5 = about 50 percent of leaves yellow and 9 = all leaves yellow. Plants scored 1 to 3 are classified as resistant.

Level of resistance to leafhopper yellowing among varieties in a test can be expressed as percentage resistant plants or as ASI. Resistant and susceptible checks should be included at least once in each replication for comparison.

By S. D. Kindler and W. R. Kehr

Evaluating spotted alfalfa aphid resistance

Methods used at Tucson, Ariz.:

Seedling tests used for evaluating spotted alfalfa aphid resistance are much like those used for pea aphid evaluations. Howe and Pesho (*24*, *25*) evaluated spotted aphid resistance in varieties at all stages of plant growth. Tests described here are similar to procedures suggested by Hackerott and others (*21*) and Klement and Randolph (*32*).

All entries are planted in galvanized flats. Each flat contains 13 rows; 8 rows are test entries, and 5 rows are susceptible checks. The rows are arranged so that each test row is adjacent to a susceptible check row. Fifty seeds are planted in each row. Each test includes four replications of each entry.

Seedlings in the unifoliolate stage are manually infested with 4 cm^3 of aphids per flat. Tests are conducted in the greenhouse at temperatures between 20° to 30°C. When all plants in the susceptible check rows are dead, counts are made of surviving seedlings in each test entry. Resistance is expressed as percent seedling survival. Test entries are compared with a resistant check included in each test.

Nielson and others (*35*) reported the existence of six biotypes of spotted alfalfa aphid in western United States. Varieties were evaluated for resistance to four biotypes by Nielson and others (*37*) using techniques described above.

By M. W. Nielson

Evaluating root-knot nematode resistance

Methods used at the Irrigated Agriculture Research and Extension Center, Prosser, Wash.:

Alfalfa varieties with high levels of resistance to root-knot nematodes are not yet available. However, sources of resistance have been identified and are being used in plant breeding programs (*27*, *43*, and *47*). Our basic evaluation procedures are patterned after those reported by Barrons (*4*) for screening beans and cowpeas in the seedling stage. However, they have been modified and are described for use with alfalfa.

Root-knot nematode resistance evaluations should be conducted in either greenhouse or growth chamber. The temperature for tests with *Meloidogyne hapla* should be 25°C (*19*) and the temperature for tests with *M. incognita* and *M. javanica* should be 30°C. A 12- to 16-hour photoperiod is recommended for tests with all three species. Seeds of test varieties are planted in rows in flats filled with steamed or fumigated soil. A row spacing of 3 cm with seeds from 1 to 2 cm apart within rows is optimum. A minimum of 100 plants per variety is recommended. We use at least four replications and include resistant and susceptible check varieties in each replication.

Two weeks after plantings, distribute an aqueous suspension of active root-knot nematodes over each flat at the rate of 600 nematodes per seedling (*10*). Twelve weeks later, carefully dig the plants, wash their roots, and examine them for root galls. Each plant is rated on a scale of 1 to 4: 1 = no galls, 2 = 1 to 10 galls, 3 = 10 to 100 galls, and 4 = > 100 galls. Care must be used when digging the plants to avoid excess root breakage and loss of galls.

Root-knot nematode resistance among varieties can be expressed as percentage resistant plants or as ASI. Only class 1 plants are considered resistant when calculating percentage resistant plants. Any galling is considered a susceptible reaction. Variety reactions are compared by statistical analyses.

Nematodes for evaluating and screening studies can be cultured on roots of tomato plants in pots in the greenhouse (other susceptible hosts also may be used). To extract nematodes from the tomato roots, discard the tops, wash the infected roots clean in water, and disinfect the surface in 10 percent chlorox for 5 minutes. The roots are place on a 20-mesh sieve screen, the screen is set in a pan of water, and the water level raised slightly above the screen surface. Newly hatched nematode larvae begin emerging in less than 24 hours, settle into the water below the screen, and continue emerging for 7 to 21 days. The water is changed daily and the nematodes stored in a refrigerator at 3°C until they are used. Because nematodes settle in standing water, they can be concentrated by siphoning off excess water. Nematode concentration can be determined by counting the nematodes in several 1 ml samples using a stereomicroscope. Nematodes should be used as soon as possible after extraction, to avoid lowering their infectivity.

A less precise, yet often useful way of inoculating with the root-knot nematodes, is to shred infected tomato roots, and mix them with the potting soil, and then plant the alfalfa seeds. A criticism of the method is that hot spots of inoculum often occur, and seedling infection may not be uniform. For preliminary work or general varietal comparisons, however, this method of inoculating may be more appealing than extracting the nematodes.

By J. H. Elgin, Jr.

Evaluating stem nematode resistance

Methods used at the Irrigated Agriculture Research and Extension Center, Prosser, Wash.:

Since O. F. Smith (*45*) compared levels of stem nematode resistance in several alfalfa varieties, several other reports have been published about screening for resistance (*5*, *17*, *18*, *20*, *23*, *44*, and *50*). All of these reports were considered during development of the methods described here. This is presently the most useful method available for accurately assessing the relative stem nematode resistance of alfalfa varieties.

Evaluation tests should be conducted either in a greenhouse or growth chamber. A 20°C temperature and 12 to 16 hour photoperiod are recommended. Seeds of test varieties are germinated on filter paper. When seedling radicals are ap-

proximately 5 mm long, they are planted in rows in metal flats filled with steamed or fumigated soil. Seedlings are spaced no closer than 2.5 cm apart in rows with rows no closer than 3 cm. Seedlings are covered with about 1.0 cm of moist soil and watered with a nematode suspension at the rate of 50 nematodes per germinating seed. Seedlings begin to emerge on the second day after planting. A stand count is made 2 weeks following planting. Second, third, and fourth inoculations are made at 2, 4, and 6 weeks after planting. An aqueous suspension of 200 active stem nematodes per plant is applied at each inoculation.

Sixteen weeks after planting, individual plants are rated according to swelling and distorting of the lower stems and crown buds caused by stem nematode attack. Rating of plants is on a 1 to 4 scale: 1 = no swelling and distortion, 2 = slight swelling and distortion, 3 = moderate swelling and distortion, and 4 = severe swelling and distortion, or death. Percentage resistant plants for each variety are obtained by considering those plants rated 1 as resistant and dividing by the number of plants present at the 2-week stand count and multiplying the quotient by 100 (table 4). Individual plant ratings may also be used to obtain an average severity score for each variety. A minimum of 100 plants per variety is recommended in each test. There should be at least five replications per test with resistant and susceptible check varieties included in each replication.

Nematodes for stem nematode studies can be obtained two ways. The most productive way is to maintain monaxenic cultures of nematodes in the laboratory. This is done by culturing the nematode on alfalfa tissue growing on a nutrient media (*12*). Nematodes are extracted from alfalfa tissue by placing the infected plant tissue and media on a 20-mesh sieve screen covered by 2-ply facial tissue. The screen is set in a pan of water so the water barely covers the plant tissue. The nematodes settle in the water below the screen, and the water is collected daily. Most of the nematodes are extracted within 48 hours. The nematodes are stored in a refrigerator at 3°C until used. Nematodes will settle in standing water and can be concentrated by siphoning off the upper water. The nematode concentration per milliliter can be determined by counting nematodes in 3 or 4, 1-ml samples using a stereomicroscope. Nematodes should be used as soon as possible after extraction to avoid lowering their infectivity.

A second way to obtain nematodes is to extract them directly from infected plant material collected from a greenhouse or field using the procedures described above. This can be disappointing because of seasonal fluctuations in nematode numbers. Not only is finding large numbers of *Ditylenchus dipsaci* in the field sometimes difficult, but this species also may be contaminated with other types of nematodes.

By J. H. Elgin, Jr.

TABLE 4.—*Evaluation of seven alfalfa varieties for stem nematode resistance 16 weeks after planting and inoculation*

Variety	Two-week stand count	Plants[1] rated 1	Resistant plants[2]
		Number	*Percent*
Ranger	98	10	10.2
Vernal	94	11	11.7
Saranac	98	39	39.8
DuPuits	97	52	53.6
Lahontan (Check)	98	60	61.2
Washoe	86	51	59.3
Apalachee	96	84	87.5

[1]Plants rated 1 to 4; 1 = no swelling and distortion, 4 = severe swelling and distortion or plant death.

[2]Percentage resistant plants = Number plants rated 1 divided by 2-week stand count.

FUTURE DEVELOPMENTS

This publication represents the first effort to compile a summary of the pest resistance evaluation for alfalfa. However, new information and evaluation procedures will continue to be developed. The need for standardizing testing and reporting procedures will continue to be important in the foreseeable future. The committee anticipates that the Alfalfa Improvement Conference will formulate procedures so that new information is available to the industry every 2 years.

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